POLYMORPHISMS OF THE SERUM PROTEINS AND THE DEVELOPMENT OF ISO-PRECIPITINS IN TRANSFUSED PATIENTS*

BARUCH S. BLUMBERG

Chief, Geographic Medicine and Genetics Section
National Institutes of Health, Bethesda, Maryland

POLYMORPHISM, according to Ford,[1] is the occurrence in the same habitat of two or more inherited forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation. This definition excludes the rare, genetically disadvantageous inherited diseases which are maintained by mutation and selected against by death or infertility. In polymorphic traits two or more of the genotypes determining variation of the trait are common in the population. Although much of the early work on polymorphism was performed on lower animals, it is now clear that humans constitute one of the most favorable species in which to study these systems. In addition, the polymorphisms provide convenient systems for the study of inherited discontinuous biochemical variation in man.

Polymorphisms are thought to arise as a result of selective differences between genotypes. For example, the sickle cell homozygote develops a severe hemolytic anemia which, under natural conditions, is usually fatal. The heterozygote, however, is at a selective advantage compared to the normal homozygote, apparently because of greater resistance to falciparum malaria, and increased fertility. As a consequence of the selection in favor of the heterozygote, the sickle gene is maintained in the population at high levels despite the elimination of genes due to the death of the homozygotes.[2] Other examples of associations of polymorphisms with exogenous disease-producing agents are known. Individuals bearing the sex-linked glucose-6-phosphate dehydrogenase deficiency (G6PD) gene (and probably at least one other trait as well)

* The *Fifth Bernadine Becker Memorial Lecture* of the New York Rheumatism Association, Inc., presented at a combined meeting of the New York Rheumatism Association and the Section on Medicine, held at The New York Academy of Medicine, November 18, 1963.

TABLE I.—SOME POLYMORPHIC TRAITS IN HUMANS

Red Blood Cell Types	*Serum Proteins*
ABO	Haptoglobin
MNS	Transferrin
P	Gamma globulin
Rhesus	Beta-Lipoprotein
Lutheran	Group-specific substance
Kell	Serum cholinesterase
Lewis	Serum esterase
Duffy	
Kidd	
Diego	
Sutter	
X-linked	

Other Cell Types	*Miscellaneous*
Hemoglobins	BAIB urinary excretion
G6PD	
Red cell phosphatase	Secretor of ABH
Red cell phosphogluconate dehydrogenase	blood group substance
White blood cell antigens	Taste of PTC
Platelet antigens	Ear cerumen character

can develop a hemolytic anemia when they eat, or are otherwise exposed to the fava bean. A similar anemia may develop if they ingest any of a large number of drugs. Individuals without this trait will not develop the anemia given the same exposure. Similarly, individuals with the inherited atypical serum cholinesterase trait can develop prolonged apnea after the administration of Suxamethonium or other related drugs (see below). It is probable that disease susceptibility related to a single inherited trait is less common than susceptibility dependent on several inherited traits. The identification of such traits may serve a useful function in identifying individuals who require special protection against specific exogenous agents.

As a consequence of disease and environmental forces, as well as other factors, a large number of polymorphisms may exist in a population. Some may be related to present selective forces, and others to forces which operated in the past, but which are no longer significant. Present gene frequencies may also result from gene mixture between populations.[3]

Table I is a list of some of the biochemical polymorphisms known in man. In the present lecture I would like to discuss the polymor-

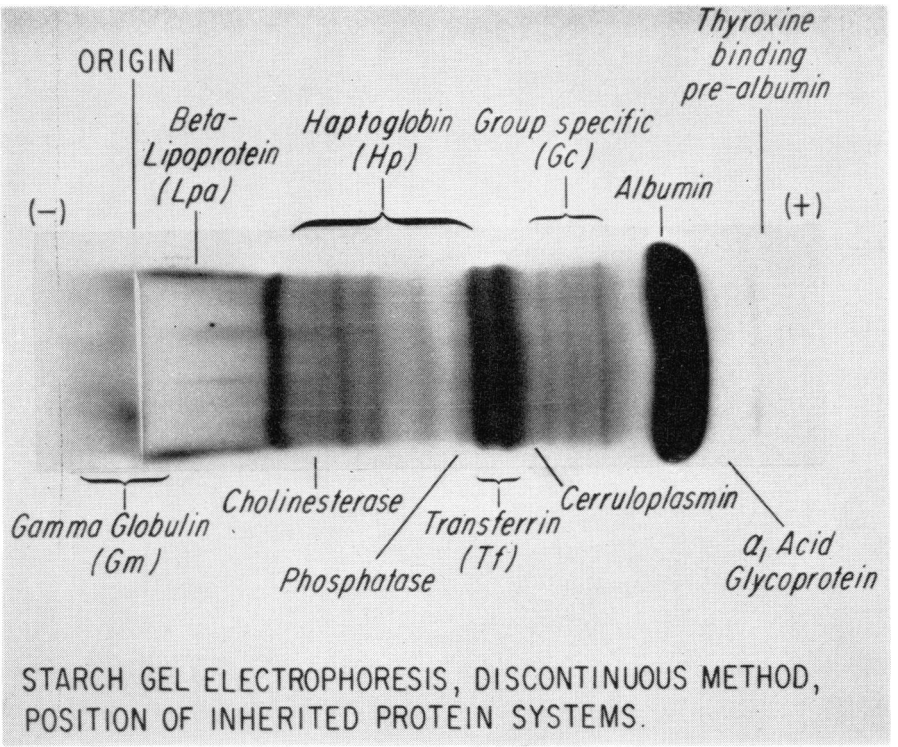

Figure 1. Photograph of starch gel electrophoresis of normal serum to illustrate the position of some of the inherited serum protein systems discussed in the text. (Adapted from reference 19.)

phisms of the serum proteins and how they relate to the problem of blood transfusion.

Serum Protein Polymorphisms (Figure 1)

The haptoglobins. The haptoglobins are a family of serum proteins which bind hemoglobin. The early studies of Jayle and his co-workers demonstrated that the haptoglobins are elevated in a wide variety of inflammatory diseases. In some illnesses associated with hemolysis, the hemoglobin released from the breakdown of the red blood cells combines with the haptoglobin to form a strong complex which appears to be removed mainly by the reticuloendothelial system. If the rate of breakdown of red blood cells is greater than the production of haptoglobin, the serum haptoglobin level will decrease, in some cases, to undetectable levels. If the hemolytic process is arrested, the haptoglobin

will be regenerated, presumably in the liver, and the serum levels may return to normal.

When starch gel is used as a supporting medium for electrophoresis, human sera can be separated according to haptoglobin type. Three major types, 1-1, 2-2, and 2-1, can be distinguished. It has been shown that in most populations these are controlled by a pair of allelic autosomal genes designated Hp^1 and Hp^2. Hp^1 homozygotes are type 1-1, Hp^2 homozygotes are type 2-2, and heterozygotes are type 2-1. Recently, Nance and Smithies[4] have shown that when purified serum haptoglobin is subjected to reductive cleavage by mercaptoethanol in the presence of 8 M urea, fast-moving and slow-moving haptoglobin variants can be distinguished. By chemical and genetic studies they demonstrated that there are three genetically determined alpha polypeptide chain variants controlled by five allelic genes Hp^{1F}, Hp^{1S}, Hp^{2FF}, Hp^{2FS}, and Hp^{2SS}. There are other relatively rare genes (i.e., Hp^{2M}) which control uncommon haptoglobin phenotypes.

Allison, Blumberg, and apRees[5] reported that approximately 30 per cent of a West African population had insufficient haptoglobin to permit typing by the starch gel method. Since this original description ahaptoglobulinemia and hypohaptoglobulinemia have been detected in other Africans, in U. S. Negroes, and in some European and Asiatic populations. There is good evidence that some forms of ahaptoglobinemia or hypohaptoglobinemia are inherited,[6] but some cases are due to the hemolysis of red blood cells and subsequent loss of haptoglobin in a variety of hemolytic diseases.

The transferrins. The transferrins (or siderophilins) are a group of serum proteins which bind iron. They can be identified by their mobility on starch gel electrophoresis and by the use of radioactive iron and autoradiography. The phenotypes are controlled by a series of apparently allelic genes, Tf^C, Tf^{BO}, Tf^{B1}, etc. The most common phenotype in U. S. and European population is type C, which corresponds to the genotype Tf^C/Tf^C. The slow-moving D variants have been found in African, American Negro, Australian aboriginal, Chinese, and other populations. Fast-moving types are occasionally found in white, Asiatic, and other populations. In recent years a large number of variants with only minute differences in electrophoretic mobility have been detected. To date no associations with particular illnesses have been found, and there does not appear to be a significant difference in the amount of

iron bound by each of the phenotypes. It has been stated that the transferrins have an inhibitory effect on virus multiplication, but it is not clear if this effect has any biological importance.

Gamma globulin groups.[7] Inherited differences in the gamma globulins were detected as a result of a chance observation made while using an immunological test for rheumatoid arthritis. The serum of many patients with rheumatoid arthritis has a gamma globulin of high molecular weight called "rheumatoid factor," which agglutinates blood cells or other particles coated with specially prepared serum gamma globulin. Grubb[8] used a system in which Rh+ human red blood cells were coated with an incomplete anti-Rh antibody. Some sera of patients with rheumatoid arthritis would agglutinate these particles. Grubb found that a gamma globulin present in the serum of some, but not all, normal individuals could inhibit the agglutination reaction. The inhibiting material was inherited in simple Mendelian fashion. Individuals homozygous or heterozygous for a dominant gene Gm^a were inhibitors. Those homozygous for all alternate allele *Gm* did not have inhibiting material in their serum. Using different combinations of coated red blood cells and sera of rheumatoid arthritis patients, different genetically controlled gamma globulins have been detected. Several of them are controlled by a series of allelic genes at the same locus (Gm^a, Gm^b, Gm^x, etc.), and others by genes at a second locus (Inv^a, Inv^b). There is as yet no information on the nature of the physiological or pathological conditions associated with this trait, although it may be connected in some way with the immune process.

Genetic variations of human serum phosphatase. Using starch gel electrophoresis, Arfors *et al.*[9] have shown that there are at least two types of human serum phosphatases. All human sera have one phosphatase band which migrates with a mobility slightly slower than serum transferrin. Some individuals, in addition, have a slower moving phosphatase band. On this basis, human sera were classified in two groups: Group I with a single phosphatase band and group II with two bands. Twin studies were highly consistent with the hypothesis that these types are inherited, but the mode of inheritance is not known. The authors also found a strong association with the Lewis blood group system, and they suggested that one serum phosphatase zone may represent the Le^b substance or a complex with the Le^b substance.

Polymorphism of α_1-acid glycoprotein. Alpha$_1$-acid glycoprotein is

a serum protein with a high (40%) carbohydrate content. It corresponds with the slow pre-albumin band on starch gel electrophoresis (Fig. 1). Schmidt and his associates[10] developed a technique for isolating large quantities of this protein from human serum or plasma. The isolated material was concentrated and subjected to starch gel electrophoresis. In comparing material isolated from different individuals, they found different patterns containing varying numbers of bands. Preliminary twin studies indicate that these patterns are inherited.

Group-specific components. Hirschfeld[11] has described a variation in the serum α-globulin which is detected by immunoelectrophoresis and is inherited in simple Mendelian fashion. The α_2-globulin involved is in the postalbumin region on starch gel electrophoresis.[12] Differences in mobility of these bands were noted by Smithies in his early studies using vertical starch gel electrophoresis. No associations with disease have been detected using this system.

Serum cholinesterase.[13] There is a gene (whose frequency in the populations tested is approximately 0.02) which determines the presence of an atypical serum cholinesterase. Individuals who are homozygous for this gene have only the atypical enzyme and are unable to inactivate the muscle relaxant succinylcholine (Suxamethonium) and related drugs with the same rapidity as individuals who are homozygous for the normal gene. When succinylcholine was administred to individuals homozygous for the atypical gene, they developed a prolonged apnea. Heterozygotes have both typical and atypical enzymes. Since the homozygotes are easily identifiable by means of a simple spectrophotometric test requiring less than 0.1 ml. of serum, it is quite practical to pre-test individuals receiving muscle relaxants. This is particularly important in shock therapy where patients often receive a prolonged course of muscle relaxants during their treatment. Other drugs which probably are also differentially metabolized by the typical and atypical enzyme include procaine hydrochloride (Novocain), physostigmine (Eserine), and chlorpromazine. It is, of course, unlikely that the differences in drug susceptibility are responsibile for the maintenance of the polymorphism. However, a number of solanaceous plants contain an inhibitor of cholinesterase. Potato peels, for instance, contain an inhibitor which can distinguish between the three cholinesterase phenotypes. It should be emphasized that under normal conditions the atypical enzyme homozygotes are quite normal; they can only be distinguished from the other

TABLE II.—REACTORS WITH ANTISERA IN DIFFERENT POPULATIONS

	C. de B.		*New York*		*J. B.*		*A. Di B.*		*S. L.*	
	No.	*% Reactor*	*No.*	*% Reactor*	*No.*	*% Reactor*	*No.*	*% Reactor*	*No.*	*% Reactor*
Whites (U.S.A.)	120	59	164	100	168	98	144	77	157	97
Micronesians (Rongelap)	194	98	185	45	54	98	45	24	50	24
Naskapi-Montagnais Indians (Labrador)	234	97	103	85	234	93	91	73	93	95

phenotypes when the appropriate drugs are administered or if the cholinesterase types are determined biochemically.

Isoantibodies in transfused patients. The existence of many polymorphisms of serum proteins makes it highly likely that a transfused patient would receive serum proteins slightly different from his own. For example, an individual of haptoglobin type 1-1, which occurs in about 15 per cent of the normal U. S. white population, has 85 of 100 chances of receiving plasma containing haptoglobin -2, if the donor is also U. S. white. Similar possibilities of receiving a different protein occur with many of the other systems. If this is so, then multiply transfused patients would often receive plasma containing "foreign" protein, albeit from the same species. In a study of transfused sera using the relatively insensitive technique of double diffusion in agar gel, a serum from a patient (C. de B.) was found to contain a precipitin which reacted with a protein present in the serum of some but not all normal individuals. The isoprecipitin did not react with any of the known polymorphic proteins, but was directed against a low density beta-lipoprotein. This technique revealed a "new" polymorphic system.[14] The ability of a serum to show a precipitin reaction with the human anti-human antiserum was found to be inherited. Individuals homozygous or heterozygous for a gene Ag^{A} reacted with antiserum C. de B. and individuals homozygous for its alternate recessive allele did not react. Further studies were initiated using the sera of other transfused patients and more than 20 sera containing isoprecipitins were found. In a study of 11 of these, at least 5 and probably 7 different specificities were identified.[15] Preliminary family studies with two of

TABLE III.

	C. de B.			*New York*			*J. B.*		
	Total	% *Reactors*	*?*	*Total*	% *Reactors*	*?*	*Total*	% *Reactors*	*?*
Coronary Artery Disease	68	60.3	1	67	88.1	3	57	75.4	13
Control	66	59.1	1	66	77.3	2	56	76.8	11
Diabetes	208	71.6	0	205	96.6	2	205	97.1	2
Control	166	55.4	1	159	98.1	2	154	94.8	5
Rheumatic Fever	165	83.6	0	37	100	0	35	100	0
Control	152	82.2	0	34	100	0	34	100	0

Reactions of three anti-lipoprotein antisera with sera from diseased patients and controls. The coronary artery disease and rheumatic fever patients and their controls were U. S. Whites and the diabetes patients and their controls were U. S. Negroes. The number of sera which could not be typed are shown in the column headed by a question mark. These studies are described in greater detail elsewhere.[17]

these suggest that they are inherited, and it is likely that the other specificities are inherited as well. Most of the antigenic specificities so far studied are common in most populations (see Table II). It appears that most individuals have several inherited antigenic specificities on these lipoproteins.

The isoprecipitins against serum lipoproteins are quite common in multiply transfused individuals. Approximately one third of thalassemia patients and 10 per cent of other patients who receive 35 or more transfusions will develop detectable isoprecipitins. The reasons for the higher frequency in thalassemia patients are not clear.

Patients who develop an isoantibody usually require additional transfusions, and this could presumably lead to antigen-antibody interactions *in vivo* similar to those studied in animals.[16] Approximately 15 patients have been observed for varying lengths of time after the isoprecipitin was discovered. In all cases, the patients have needed frequent transfusions, very often with blood known to contain the proteins which *in vitro* form precipitin bands with the patient's isoprecipitin. In none of these cases has it been possible to associate with the isoprecipitin an acute, severe transfusion reaction. It has not been possible as yet to evaluate the long-term effect of continued transfusions.

The selective or disease factors, if any, associated with this polymorphism are unknown. As a preliminary investigation of possible

disease associations, two entities known to be associated with abnormalities of the lipoproteins were studied. In addition, the sera of patients with rheumatic fever, collected for other reasons, were also studied. The frequency of reaction of these patients and controls with three of the antisera are shown in Table III.[17] There is a significantly higher number of reactors with the C. de B. antisera in the diabetic as compared to the control group. No such differences are detected for the other groups. This finding is interesting, but before any importance can be attached to it, this study will have to be repeated in other populations. If it proves to be reproducible, then the higher frequency of reactors might be due to some feature of the disease which converts apparent non-reactors into detectable reactors, or it may possibly be related to an inherited susceptibility factor.

In addition to the isoprecipitins which react with lipoproteins, an isoprecipitin has been found which reacts with an as yet unidentified protein which does not appear to have a large amount (or in some cases, any) lipid. The isoprecipitins are found in multiply transfused hemophilia patients. The protein with which they react is very rare in populations of Western origin, but it is not uncommon in Micronesians, Vietnamese, Formosans and Australian aborigines. The reacting antigen is also quite common in patients with leukemia, and the significance of this is currently under study.

In addition to these precipitating antibodies, Allen and Kunkel[18] have found agglutinating antibodies against gamma globulin (anti-Gm antibodies) in the sera of many multiply transfused thalassemia patients. It is not unlikely that, using the appropriate techniques, antibodies against a very large number of serum proteins will be detected. These may be the cause of some of the transfusion reactions which cannot be ascribed to interaction with the formed elements of the blood.

Summary

The concept of polymorphism is discussed and known biochemical polymorphisms in man are listed. The serum protein polymorphisms and the formation of isoprecipitins in multiply transfused human patients are described.

REFERENCES

1. Ford, E. B. Polymorphism in plants, animals and man, *Nature (London)* *180*:1315-19 (Dec. 14), 1957.
2. Allison, A. C. Aspects of polymorphism

in man, *Cold Spring Harbor Symposia on Quantitative Biology 20*:239-55, 1955.
3. Blumberg, B. S. Inherited susceptibility to disease. Its relation to environment, *Arch. Environ. Health (Chicago) 3*:612-36, 1961.
4. Nance, W. E. and Smithies, O. New haptoglobin alleles: A prediction confirmed, *Nature (London) 198*:869-70 (June 1), 1963.
5. Allison, A. C., Blumberg, B. S. and apRees, W. Haptoglobin types in British, Spanish Basque and Nigerian populations, *Nature (London) 181*:824-25 (March 22), 1958.
6. Giblett, E. R. and Steinberg, A. G. Inheritance of serum haptoglobin types in American Negroes: Evidence for a third allele, Hp^{2M}, *Amer. J. Hum. Genet. 12*: 160-69, 1960.
7. Fundenberg, H. and Franklin, E. C. Human gamma globulin. Genetic control and its relation to disease, *Ann. Intern. Med. 58*:171-80, 1963.
8. Grubb, R. Relationship between blood group serology and rheumatoid arthritis serology. Serum protein groups, *Vox Sang. 2*:305-12, 1957.
9. Arfors, K.-E., Beckman, L. and Lundin, L.-G. Genetic variations of human serum phosphatases, *Acta Genet. (Basel) 13*: 89-94, 1963.
10. Schmid, K. and others. Studies on the structure of α_1-acid glycoprotein and the partial resolution and characterization of its variants, *Biochemistry (Wash.) 1*:959-66, 1962.
11. Hirschfeld, J. Immune-electrophoretic demonstration of qualitative differences in human sera and their relation to the haptoglobins, *Acta Path. Microbiol. Scand. 47*:160-68, 1959.
12. Cleve, H., Prunier, J. H. and Bearn, A. G. Isolation and partial characterization of the two principal inherited group-specific components of human serum, *J. Exper. Med. 118*:711-26, 1963.
13. Kalow, W. and Gunn, D. R. Some statistical data on atypical cholinesterase of human serum, *Ann. Hum. Genet. 23*: 239-50, 1959.
14. Blumberg, B. S., Bernanke, D. and Allison, A. C. A human lipoprotein polymorphism, *J. Clin. Invest. 41*:1936-44, 1962.
15. Blumberg, B. S. and others. Multiple antigenic specificities of serum lipoproteins detected with sera of transfused patients. *Vox Sang. 9*:128-45, 1964.
16. Weigle, W. D. Fate and biological action of antigen-antibody complexes, *Advances in Immunology 1*:283-317, 1961.
17. Blumberg, B. S., Ledbetter, B. B. and Visnich, S. Inherited antigenic differences in serum beta-lipoproteins. Studies in association with disease, *Amer. J. Public Health*, 1964. In press.
18. Allen, J. C. and Kunkel, H. G. Antibodies to genetic types of gamma globulin after multiple transfusions, *Science 139*:418-19, 1963.
19. Robinson, J. C. and Pierce, J. E. Prevention of background staining of starch gels, *Amer. J. Clin. Path. 40*:588-90, 1963.